Next-generation sequencing for MRD assessment in acute myeloid leukemia

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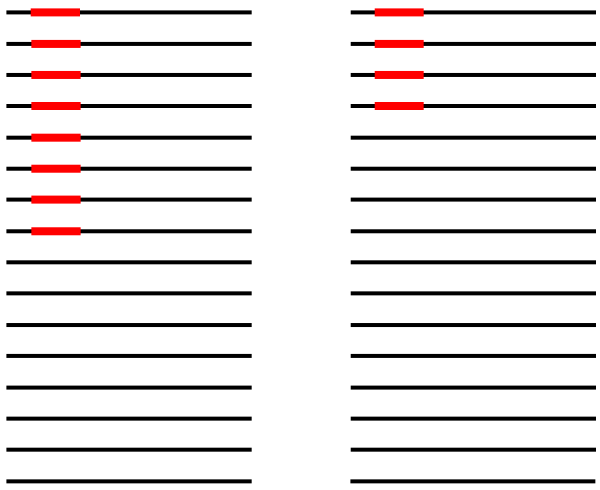
Outline

1. Overview of technology
2. MRD assessment by 454 sequencing
3. MRD assessment with Illumina sequencer
4. Stability of molecular markers from diagnosis to relapse
5. MRD assessment by NGS in lymphoid neoplasias

Next generation sequencing for MRD monitoring

Concept

Patient DNA is sequenced at the clonal level

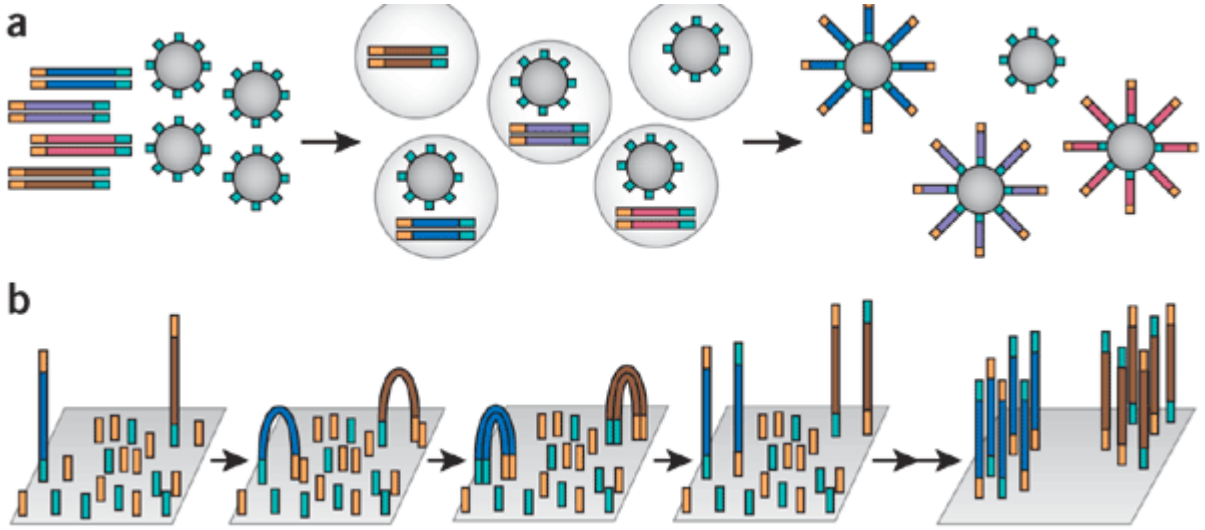


Quantification of allelic burden:

Counting the number of wildtype sequences and mutated sequences

Next generation sequencing platforms

- Unlike for RT-PCR, there is no need for a sequence specific probe – increases flexibility
- Technology platforms:
  - 454 pyrosequencing – based on emulsion PCR
  - Illumina Genome Analyzer – based on bridge PCR
  - AB SOLiD – based on emulsion PCR
  - Ion torrent – semiconductor technology, pH change



Comparison of NGS systems (2012)

	454 GS FLX	HiSeq 2000	SOLIDv4	Ion Torrent
Chemistry	Synthesis Pyrosequencing	Synthesis Reversible termination	Ligation sequencing	Synthesis H^+ detection
PCR Amplification	Emulsion PCR on beads	Bridge PCR	Emulsion PCR on beads	Emulsion PCR on beads
Reads	1 M	3 G	1.4 G	5 M
Read length	700 nt	100 nt	75 nt	200 nt
Error rate	1%	0.1%	0.01%	1%
Cost per Mb*	10 $	0.07 $	0.13 $	0.93 $

* Conventional Sanger sequencing: 2400 $

Liu L. et al. J Biomed Biotechnol 2012

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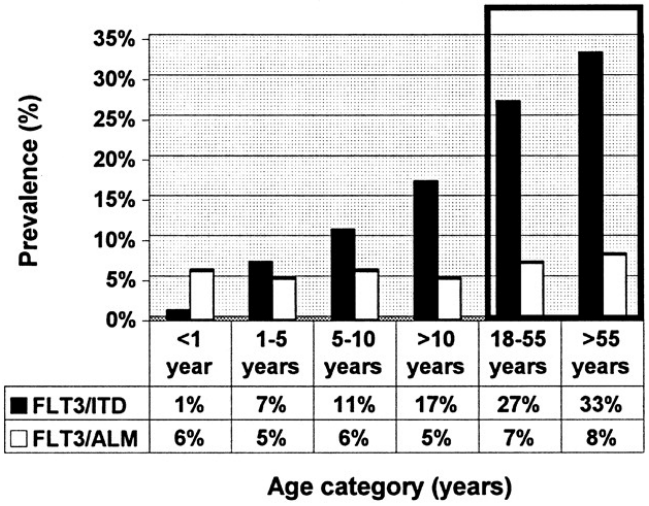
Rationale for FLT3-ITD, NPM1 and DNMT3A MRD assessment

- FLT3-ITD mutations are variable from patient to patient, and even within a patient more than one clone may be present at a time
- Negative prognostic influence:
  - Insertion in ß1-sheet (vs. JMD)
  - High allelic ratio ITD vs wildtype
  - longer insertions

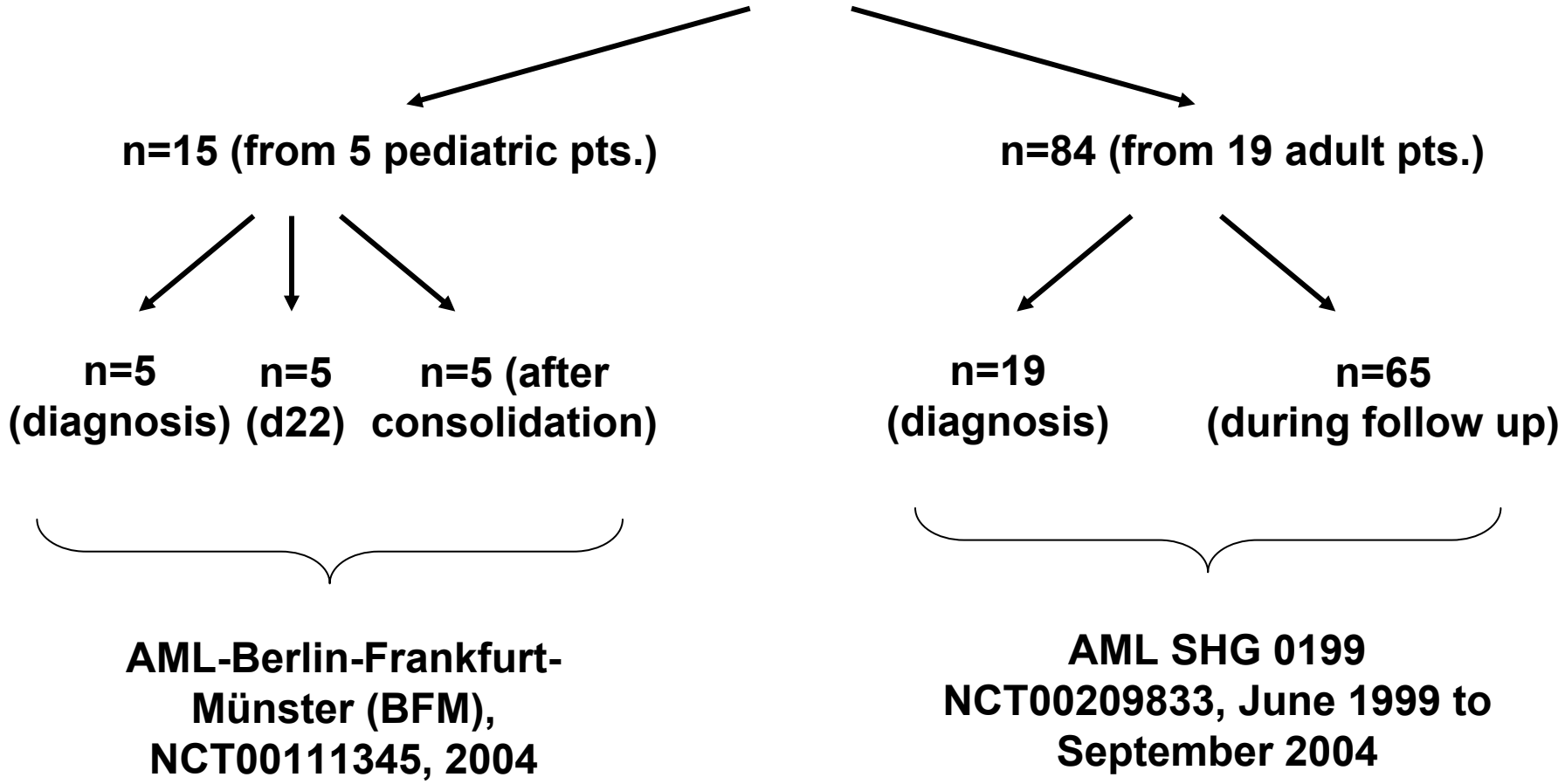


	<1 year	1-5 years	5-10 years	>10 years	18-55 years	>55 years
■ FLT3/ITD	1%	7%	11%	17%	27%	33%
□ FLT3/ALM	6%	5%	6%	5%	7%	8%

- Resistance against FLT3 inhibitors may occur from outgrowth of FLT3-ITD negative clones or resistant FLT3-ITD clones
- NPM1 is a well established MRD target and therefore was used as a control
- DNMT3A mutations have variable locations, multiple or even individual primer/probe sets are required for RT-PCR based MRD

Meshinchi et al. Blood 2006

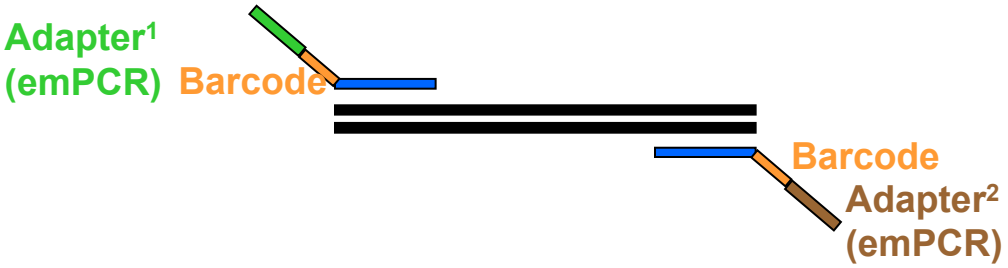
Patients: 99 samples from 24 patients



Healthy donors as controls

Methods (1)

1. Amplicon sequencing of FLT3 (328 bp) and NPM1 (536 bp) by 454 technology



A) Conventional PCR amplification

B) Clean-up with Ampure beads

C) Mix of FLT3 and NPM1 PCRs at a 1:2 ratio

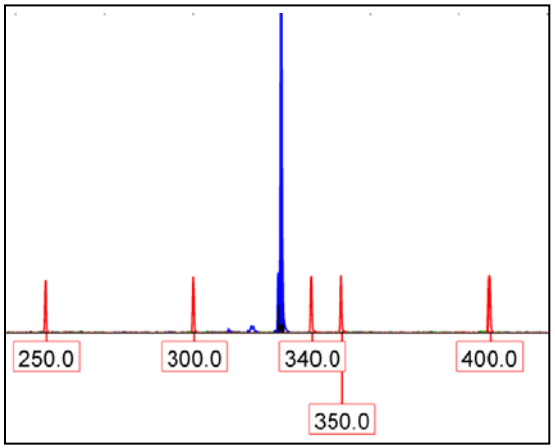
D) emPCR with Library-L chemistry

E) 454 sequencing on PicoTiterPlate, GS FLX sequencer using titanium chemistry Lib-L

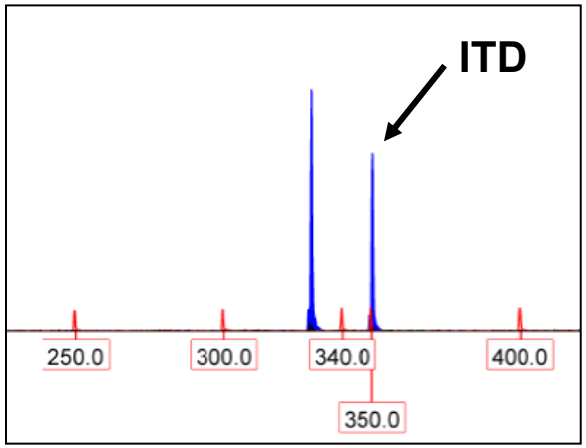
Methods (2)

2. NPM1 qRT-PCR with MutaQuant kit from Ipsogen, Marseille, France (mutation A, B, C)

3. Fragment analysis of FLT3 PCR amplicons by capillary electrophoresis (3130 genetic analyzer)



FLT3 wildtype

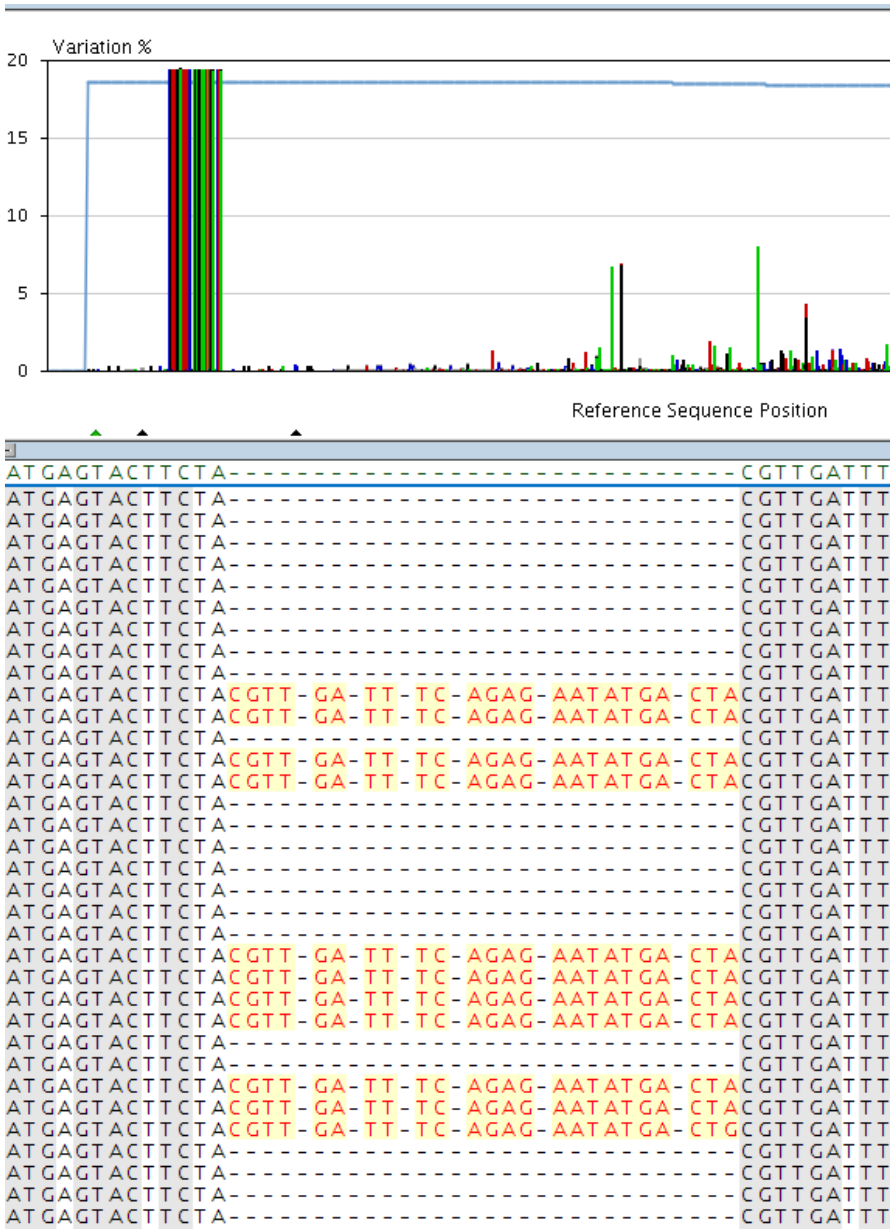


FLT3 internal tandem duplication (ITD)

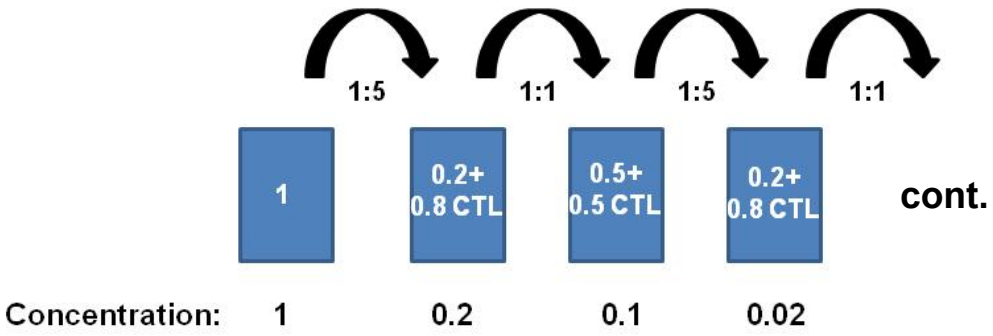
Results

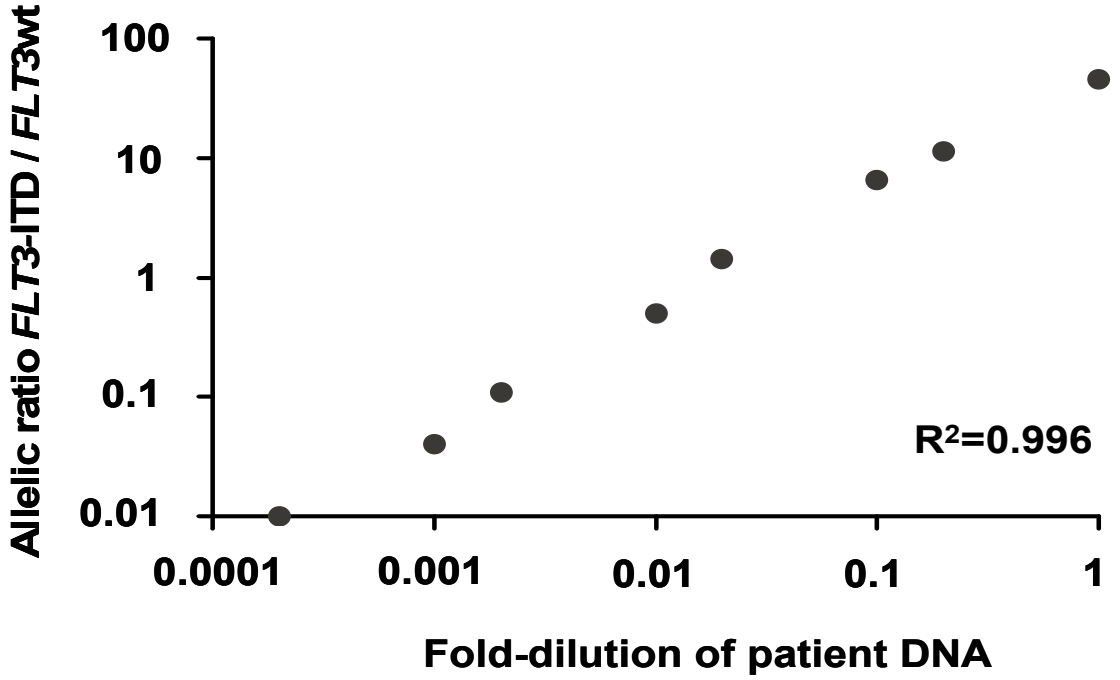
- **Total reads: 2,563,550 reads**
- **High quality reads: 1,176,171 reads**
- **FLT3 average read depth: 15,278 reads per sample (range 5,525 – 24,997)**
- **NPM1 average read depth: 7,758 reads per sample (range 393 – 18,188)**

FLT3-ITD
AVA analysis

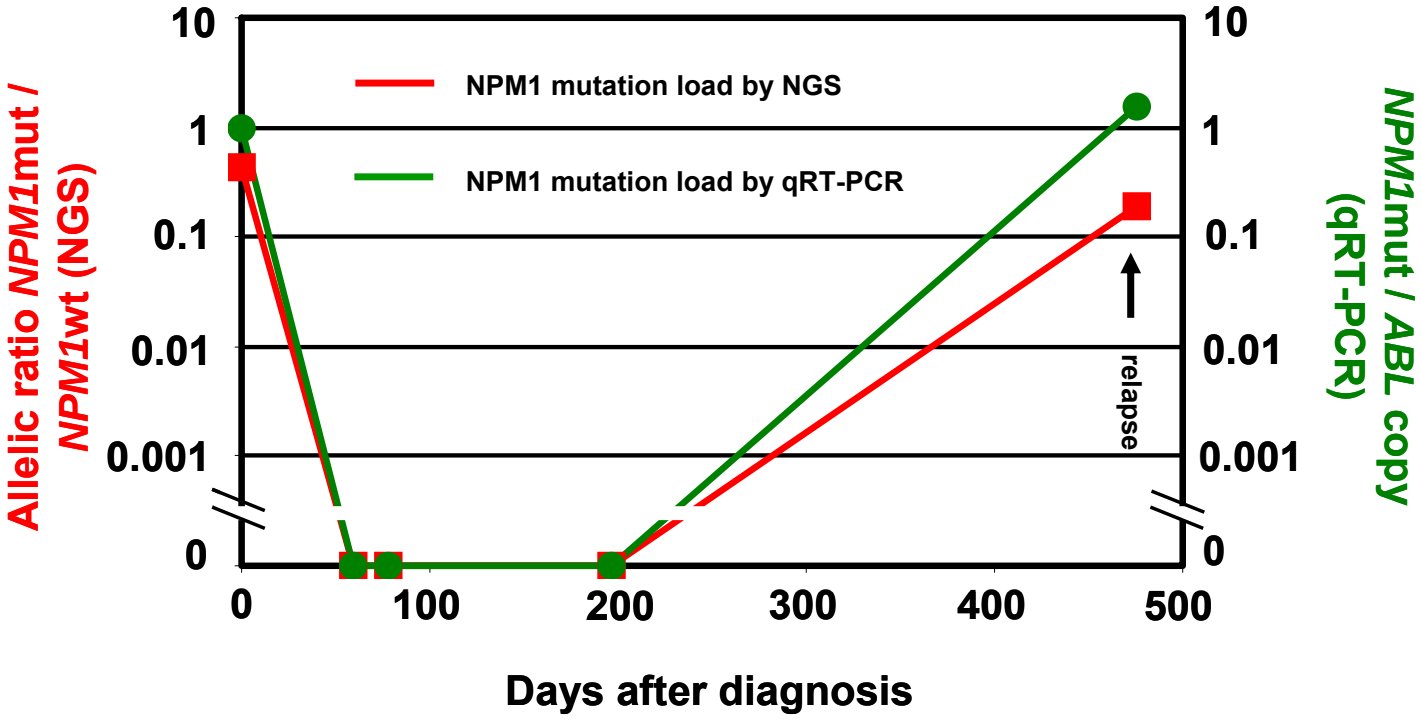


Allelic ratios can be reliably determined





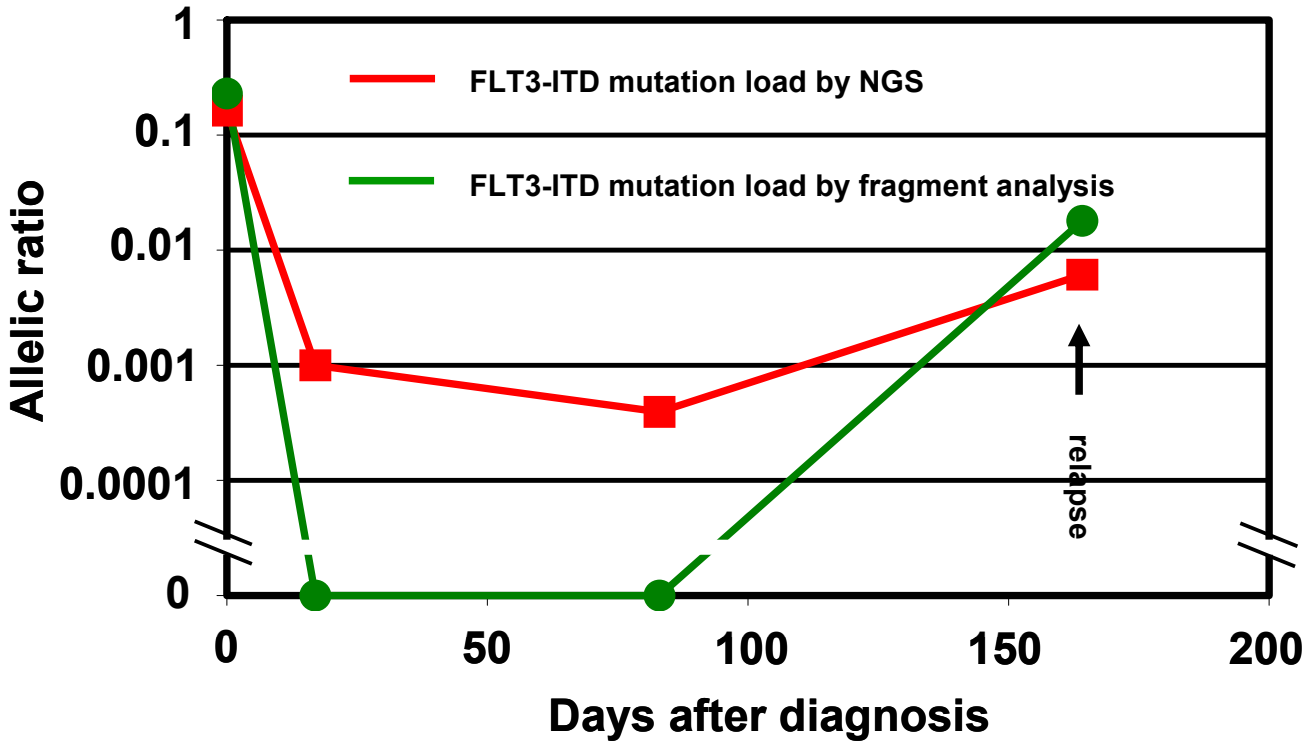
NPM1: Comparison of 454 sequencing and qRT-PCR



Mean allelic ratio at diagnosis in BM: 0.37 (range 0.29-0.46)

Concordant results between 454 and qRT-PCR: 38 / 40 samples = 95%

FLT3: Comparison of 454 sequencing and fragment analysis



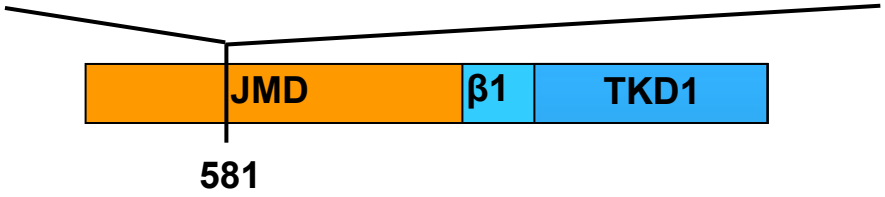
Mean allelic ratio at diagnosis in BM: NGS: 0.27 (range 0.29-0.46)
Fragment analysis: 0.4

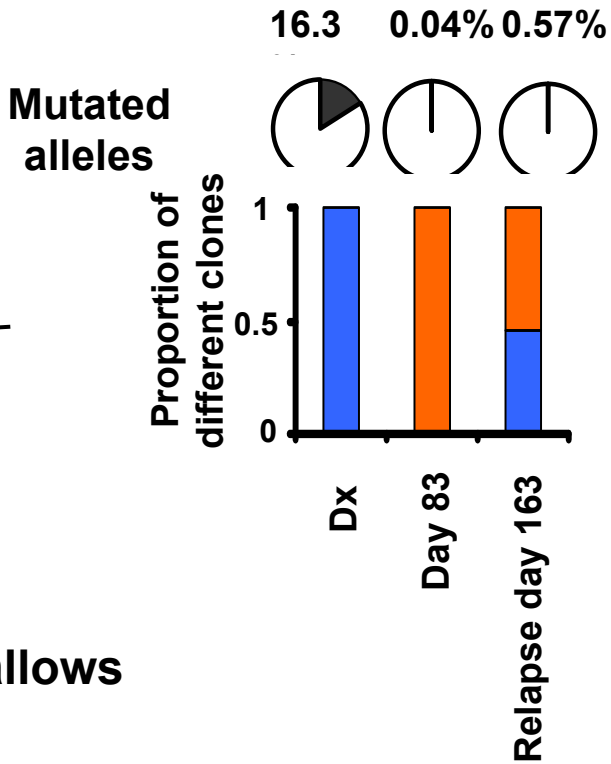
Concordant results between NGS and fragment analysis: 17 / 20 samples = 85%

Clonal dominance over time: example 1

clone 1: CGGCTCCTCAGATAATGAGTACTTCTACGTTGAT
TTCAGAGAATATGAATATGATCT (16.3%)

clone 2: TACGGTACCGGCTCCTCAGAAATGAGTACCAGT
TACAGAGAATATGAATAT (0%)





454 sequencing of FLT3-ITD mutated patients allows determination of

- **insertion site**
- **insertion length**
- **insertion sequence**
- **number of FLT3-ITD clones**
- **allelic ratio of clones**

sequentinal analyses: clonal dominance

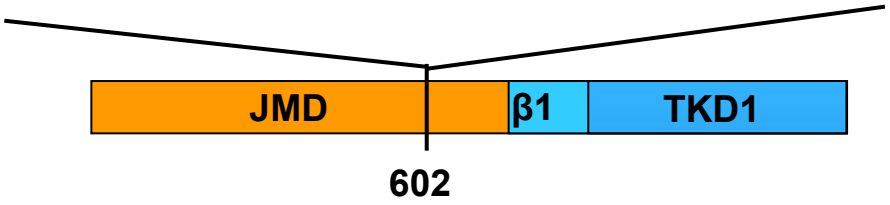
Thol et al. Genes, Chromosomes and Cancer 2012

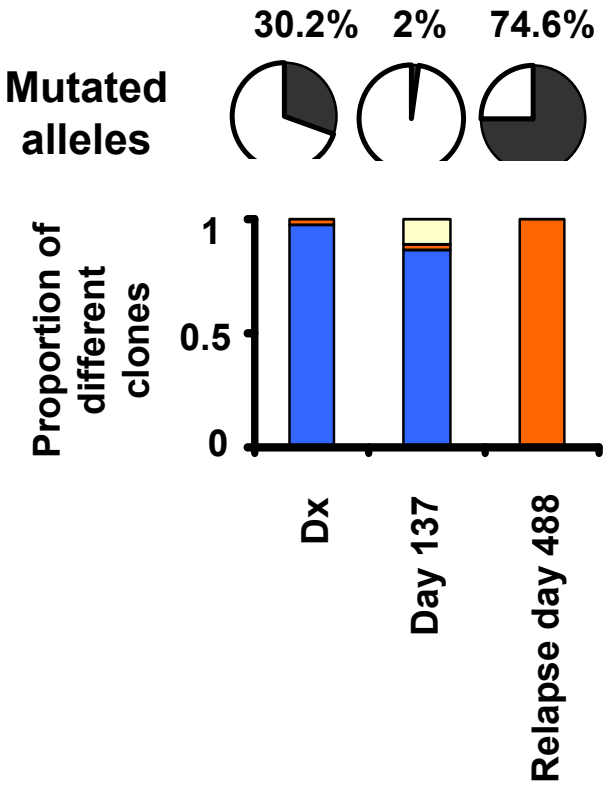
Clonal dominance over time: example 2

clone 1: GAGAATATGAATATGATCTCA (29.5%)

clone 2: CAGATAATGAGTACTTCTACGTTGATTTCAG
AGAATATGAATATGATCTCAAATGGGAGTTT
CCAAGAGAAAATTTAGAGTTTG (0.66%)

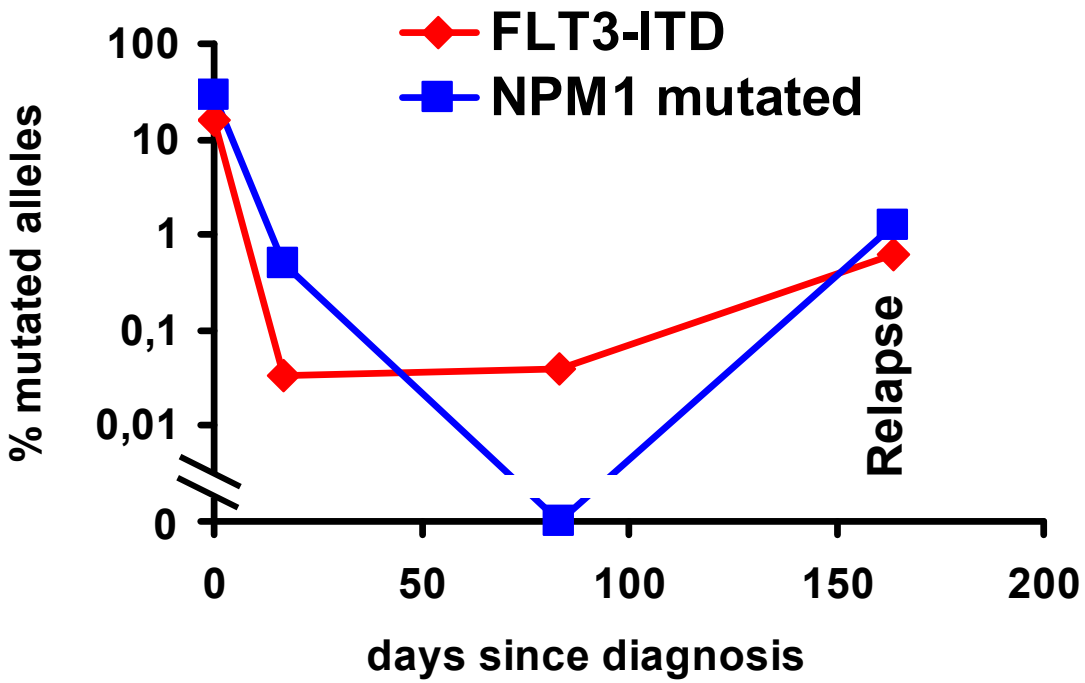
clone 3: AAGAATTAGATCTCAAATGGGAGTTTC (0%)





The minor clone dominates at relapse most likely through loss of the wildtype allele

Comparison of FLT3-ITD and NPM1 mutation in one patient

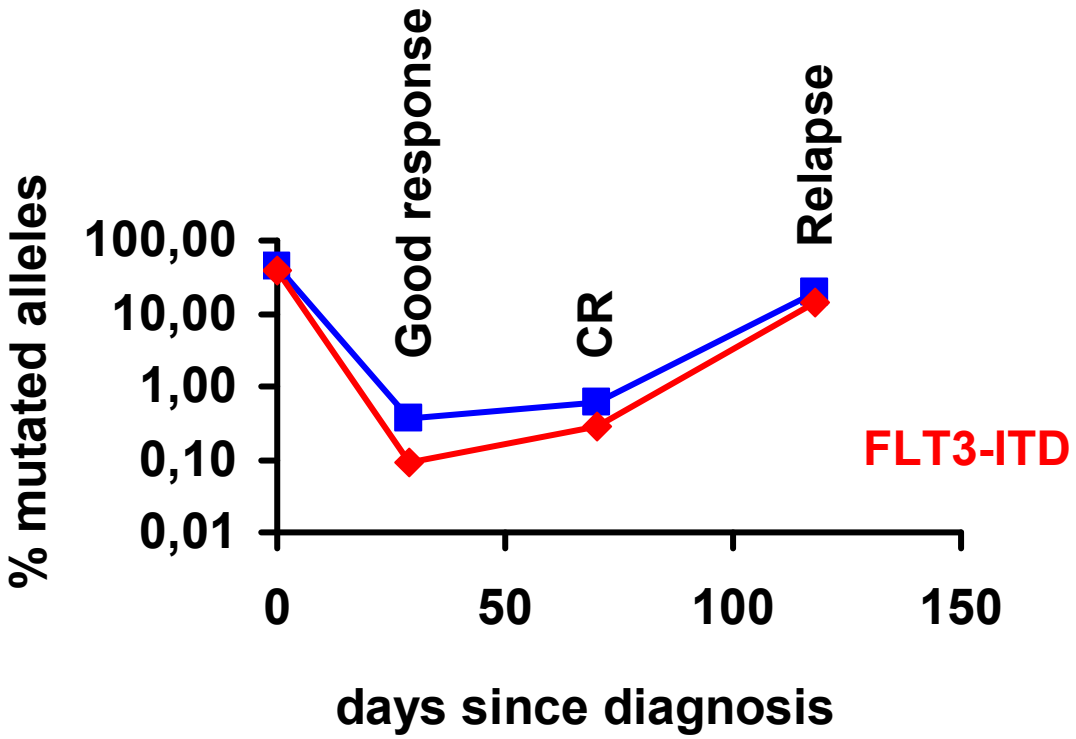


Coverage	d0	d17	d83	d164
FLT3	12,889	18,097	16,498	12,876
NPM1	7,969	8,568	13,500	16,625

DNMT3A R882H mutation – MRD by 454

Patient 1

- **47,XX, +8**
- **DNMT3A R882H**
- **FLT3-ITD**
- **NPM1 mutation**
- **NRAS mutation**

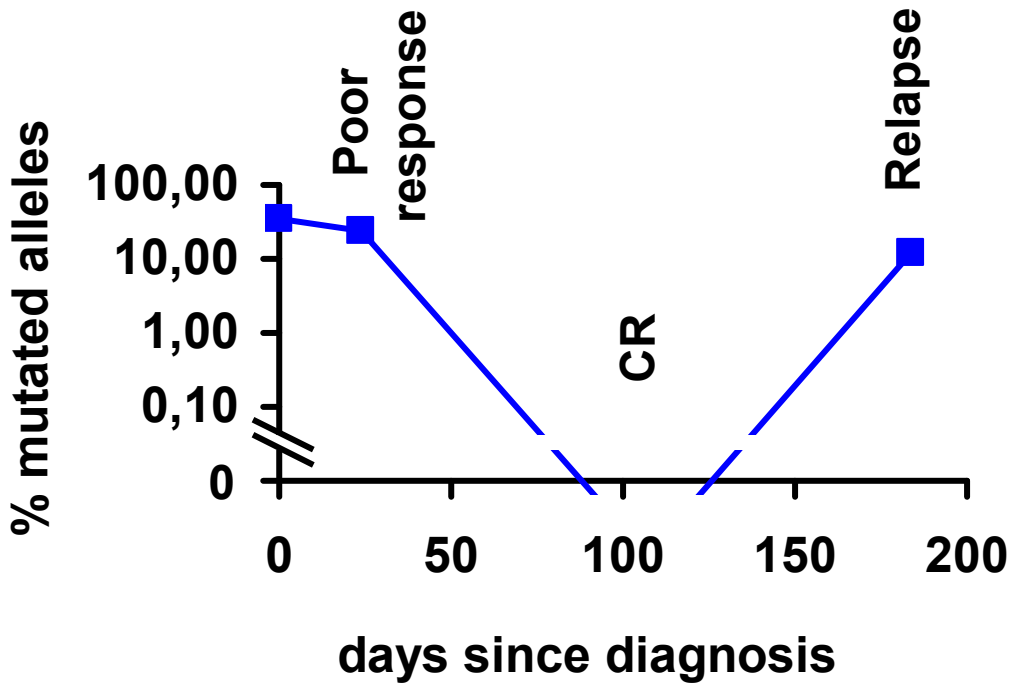


Coverage	d0	d29	d70	d118
Reads (n)	6,390	16,472	10,384	7,523
Ratio (%)	44.2	0.37	0.6	19.5

DNMT3A W893S mutation – MRD by 454

Patient 2

- **51, XY, +6,+8,+10,+13,+17**
- **DNMT3A W893S**

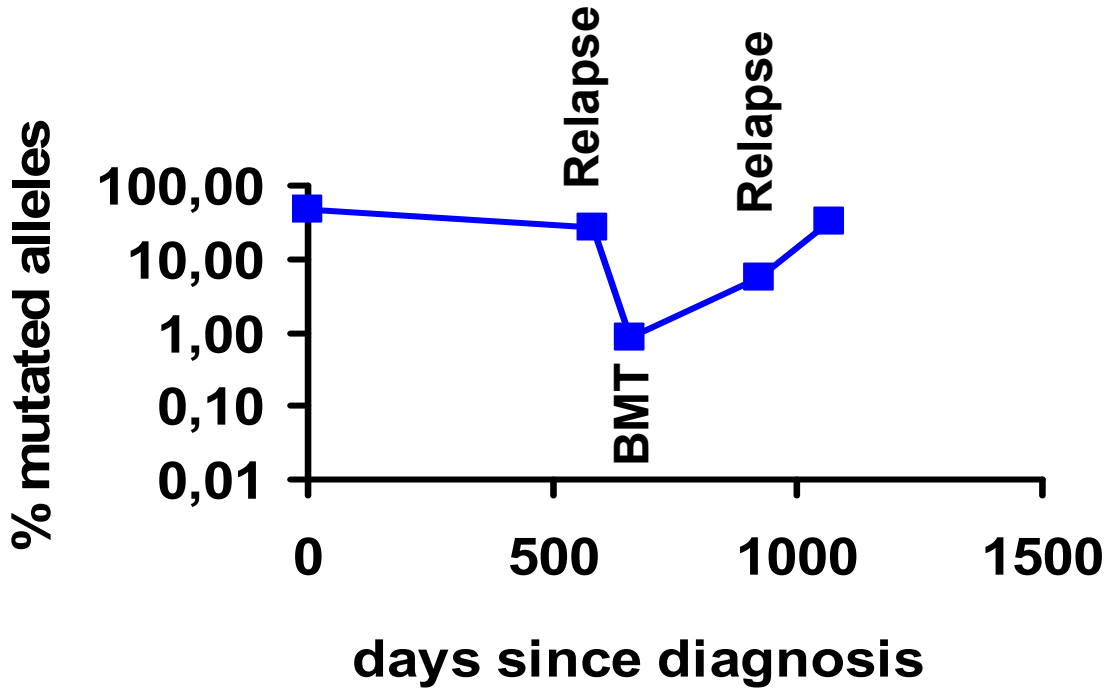


Coverage	d0	d24	d107	d184
Reads (n)	11,555	13,265	15,444	9,563
Ratio (%)	35.3	23.8	0	11.95

DNMT3A R882C mutation – MRD by 454

Patient 3

- **46,XX**
- **DNMT3A R882C**
- **NPM1 mutation**
- **TET2 mutation**
- **NRAS mutation**

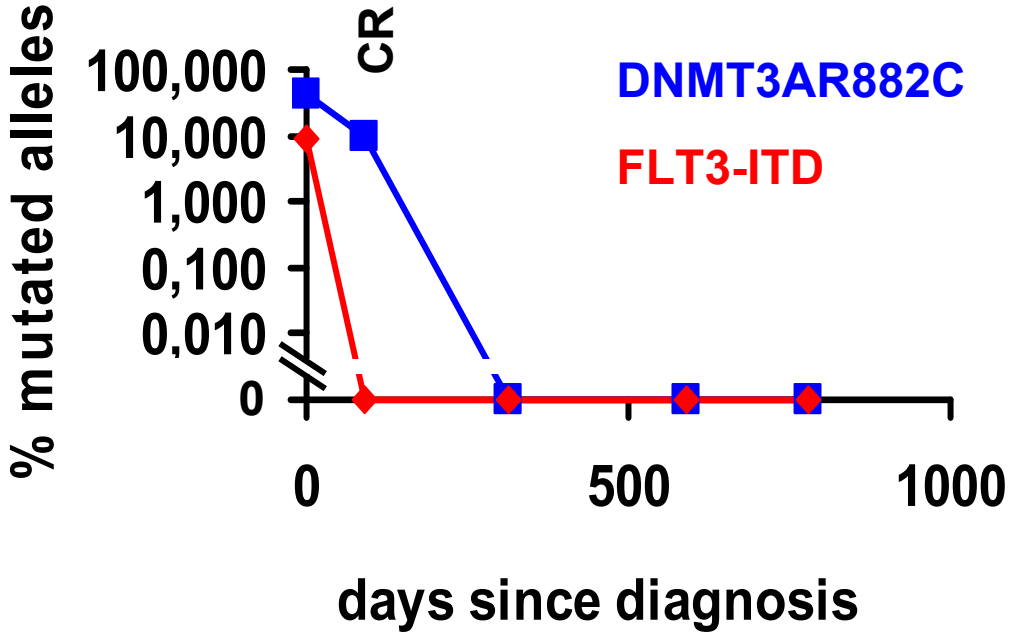


Coverage	d0	d583	d658	d922	d1066
Reads (n)	9,152	15,805	14,502	16,581	11,122
Ratio (%)	47.3	27.2	0.83	5.7	32.2

DNMT3A R882C mutation – MRD by 454

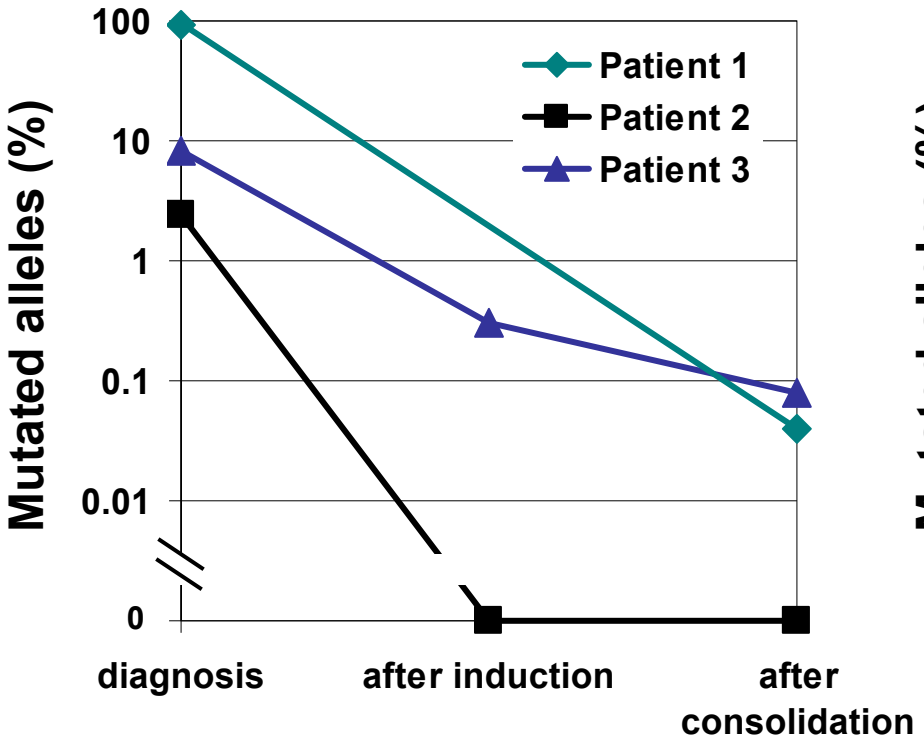
Patient 4

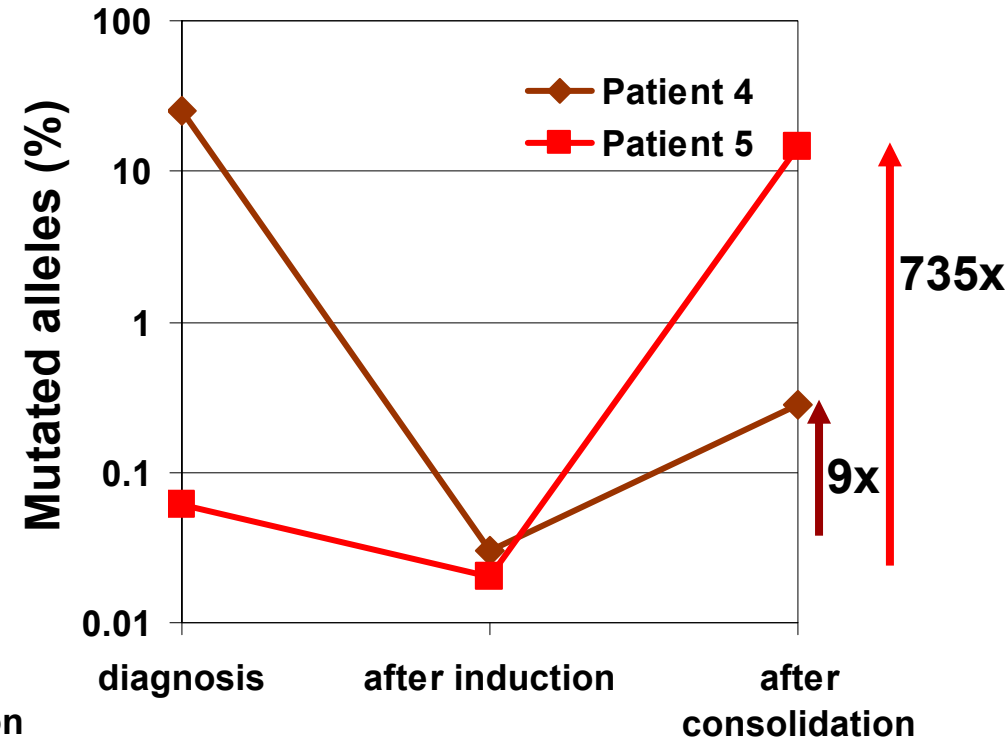
- **46,XY**
- **DNMT3A R882C**
- **NPM1 mutation**
- **FLT3-ITD**



Coverage	d0	d90	d314	d591	d779
Reads (n)	9,320	9,979	10,054	4,427	17,484
Ratio (%)	41	9.9	0	0	0

Pediatric AML: MRD monitoring for FLT3-ITD





All patients remained in remission

Patient 4: relapse 303 days after end of consolidation

Patient 5: relapse 74 days after end of consolidation

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#408 Comparison of Mutation Patterns Between Diagnosis and Relapse in 444 Patients with Acute Myeloid Leukemia Shows High Variability of Stability and Influence On Time to Relapse

Susanne Schnittger, PhD, Tamara Alpermann*, Niroshan Nadarajah, MSc*, Vera Grossmann, MSc, Christiane Eder, PhD*, Alexander Kohlmann, PhD, Annette Fasan, PhD*, Frank Dicker, PhD, Wolfgang Kern, MD, Claudia Haferlach, MD and Torsten Haferlach, MD

MLL Munich Leukemia Laboratory, Munich, Germany

Stability of molecular markers between diagnosis and relapse (1)

1. **Disease-defining markers: PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11, DEK-CAN, MLL-translocations, NUP98-fusions, NPM1, CEBPA, MLL-PTD, RUNX1**

- **A median of 12 Markers were studied in 444 patients who eventually relapsed.**
- **At least 1 disease-defining marker was present in 400 patients at diagnosis.**
- **In all patients the disease-defining marker was also present at relapse**

Stability of molecular markers between diagnosis and relapse (2)

2. Accompanying mutations: FLT3-ITD, FLT3-TKD, NRAS, KRAS, KIT, IDH1, IDH2, ASXL1, DNMT3A, TET2, WT1

- 1-5 accompanying mutations were present in 288 patients
- The diagnostic mutation pattern was stable at relapse in 55%
- At least one marker was lost at relapse in 22.7%
- At least one marker was gained at relapse in 9.5%
- FLT3-ITD was stable between diagnostic and relapse samples in 86.3%

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#788 Next-Generation Sequencing and Real-Time Quantitative PCR for Minimal Residual Disease (MRD) Detection Using the Immunoglobulin Heavy Chain Variable Region: A Methodical Comparison in Acute Lymphoblastic Leukemia (ALL), Mantle Cell Lymphoma (MCL) and Multiple Myeloma (MM)

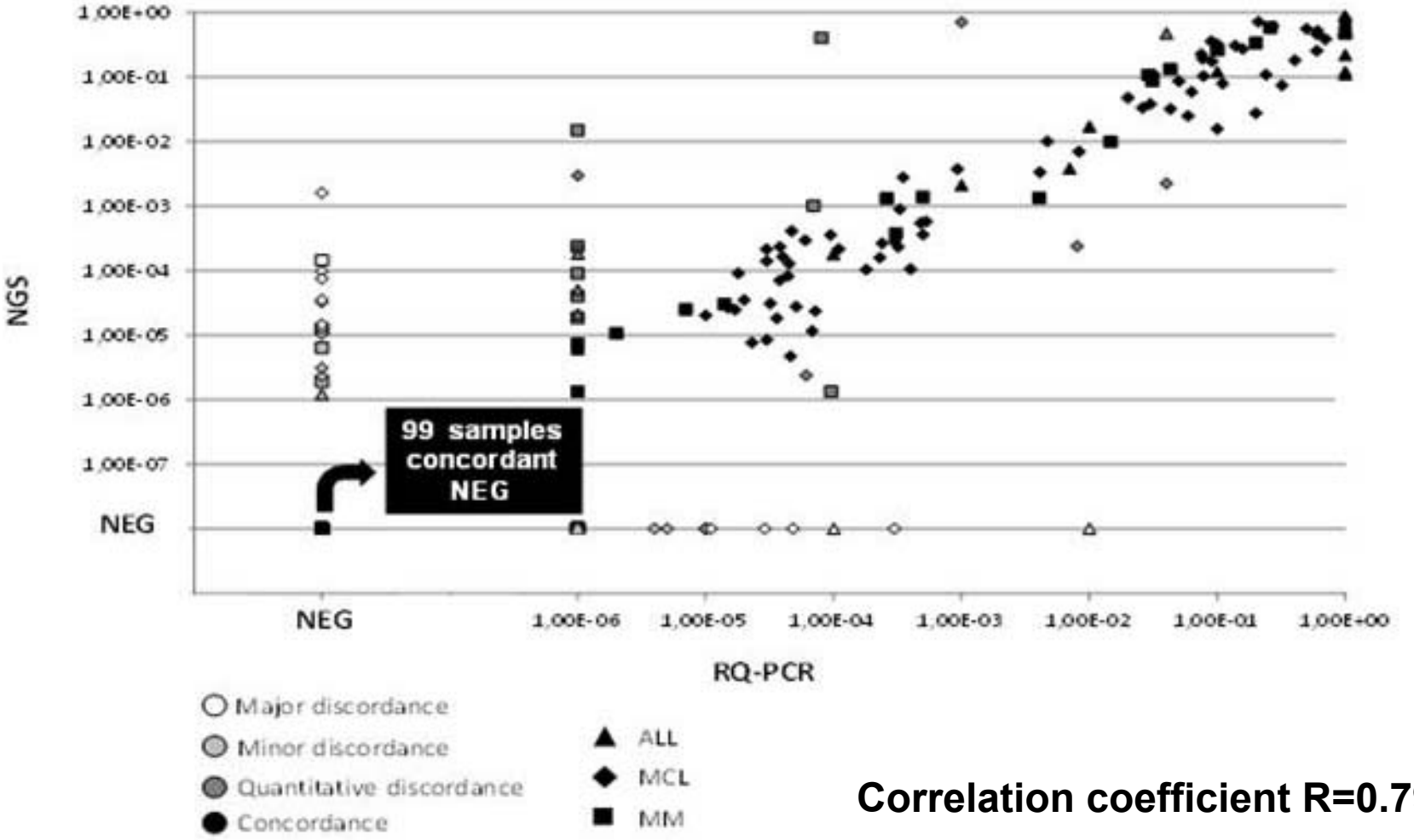
Marco Ladetto, MD1*, Monika Bruggemann2*, Simone Ferrero, MD1*, Francois Pepin3*, Daniela Drandi, PhD1*, Luigia Monitillo, PhD1*, Antonio Palumbo, MD1*, Roberto Passera, PhD4*, Mario Boccadoro, MD1, Victoria Carlton, PhD5*, Heiko Trautmann2*, Malek Faham, MD, PhD5 and Christiane Pott2*

1Division of Hematology, University of Torino, A.O. San Giovanni Battista, Torino, Italy
2Second Medical Department, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
3Sequenta Inc, San Francisco, CA
4Division of Nuclear Medicine, Statistical Consultant, University of Torino, Torino, Italy
5Sequenta, Inc., South San Francisco, CA
ASH 2012, Atlanta

Success rate of RQ-PCR and NGS MRD analysis of IGHV region

Disease	Patients	Patients evaluable PCR	Patients evaluable NGS	Patients evaluable with both tools	Patients evaluable with at least one tool	Patients not evaluable
ALL	15	15	15	15	15	0
MCL	30	22	26	22	26	4
MM	10	8	8	6	10	0
Total	55	45	49	43	51	4

Correlation analysis between RQ-PCR and NGS results



Correlation coefficient R=0.79

Conclusion

- **Next generation sequencing is a flexible and sensitive tool to detect minimal residual disease**
- **NGS allows identification of clonal composition and dominance of FLT3-ITD mutations, and can be applied to any other mutation or gene fusion**
- **The sensitivity of MRD detection is scalable depending on sequence coverage**

Conclusion

- **Use of 454 GS FLX is limited by low coverage and high cost, but seems beneficial for long insertions like FLT3-ITD**
- **MiSeq and Ion Torrent instruments have a fast turnaround time and were designed for clinical applications**
- **Clonal evolution may limit the predictive value of MRD**
- **But analysis of multiple targets in one patient may increase the predictive value**
- **Concept: screen patients for mutations at diagnosis, then follow up on multipe mutations at defined time points.**

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